FILE 'REGISTRY' ENTERED AT 14:02:44 ON 19 JAN 2001

=> S TRANSESTERIFICATION/CN

L1 0 TRANSESTERIFICATION/CN

=> S TRANSESTERIFICATION

L2 4 TRANSESTERIFICATION

=> S HYDROLASE/CN

L3 1 HYDROLASE/CN

=> D

L3 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2001 ACS
RN 9027-41-2 REGISTRY
CN ***Hydrolase (9CI)*** (CA INDEX NAME)
OTHER NAMES:
CN Adrelase
CN Hydrolytic enzymes
MF Unspecified
CI MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, CEN, CIN, CSNB, EMBASE, IFICDB, IFIPAT, IFIUDB, PIRA, PROMT, TOXLINE, TOXLIT, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
2167 REFERENCES IN FILE CA (1967 TO DATE)
38 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
2169 REFERENCES IN FILE CAPLUS (1967 TO DATE)

=> S SUBTILISIN/CN

L4 1 SUBTILISIN/CN

=> D

L4 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2001 ACS
RN 9014-01-1 REGISTRY
CN ***Subtilisin (9CI)*** (CA INDEX NAME)
OTHER NAMES:
CN Alcalase
CN Alcalase 0.6L
CN Alcalase 2.4L
CN Alcalase 2.5L
CN ALK-enzyme
CN Bacillopeptidase A
CN Bacillopeptidase B
CN Bacillus subtilis Alkaline proteinase
CN Bacillus subtilis alkaline proteinase
CN Bioprase
CN Bioprase AL 15
CN Bioprase APL 30
CN Bioprase Conc
CN Bioprase PN 4
CN Bioprase SP
CN Bioprase SP 20
CN BLAP 260
CN BLAP S
CN Chirazyme P 1
CN ChiroCLEC-BL
CN Colistinase
CN E.C. 3.4.21.14
CN E.C. 3.4.21.62
CN E.C. 3.4.4.16
CN Esperase
CN Esperase 8.0L
CN Evertase 24 LDP
CN Extretex
CN Genenase

CN Genenase I
CN Kazusase
CN Maxacal
CN Maxacal CX600K
CN Maxatase
CN Nagarse
CN Opticlean
CN Orientase 10B
CN Peptidase, subtilo-, A
CN Prosperase L
CN Protease S
CN Protease VIII
CN Protease XXVII
CN Proteinase, Bacillus subtilis alkaline
CN Protin A
CN Protin A 3L
CN Purafect L
CN Savinase
CN Savinase 12 T
CN Savinase 12.0T
CN Savinase 12T
ADDITIONAL NAMES NOT AVAILABLE IN THIS FORMAT - Use FCN, FIDE, or ALL for DISPLAY
DR 12626-20-9, 12770-87-5, 9028-05-1, 9031-73-6, 9045-36-7, 9063-47-2, 9067-41-8, 2392-42-9, 148093-32-7, 196414-34-3
MF Unspecified
CI COM, MAN
LC STN Files: AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAOLD, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHEM, CSNB, DDFU, DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, MSDS-OHS, NAPRALERT, NIOSHTIC, PROMT, RTECS*, TOXLINE, TOXLIT, ULIDAT, USPATFULL
(*File contains numerically searchable property data)
Other Sources: DSL**, EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
4105 REFERENCES IN FILE CA (1967 TO DATE)
290 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
4110 REFERENCES IN FILE CAPLUS (1967 TO DATE)

FILE 'CAPLUS' ENTERED AT 14:04:40 ON 19 JAN 2001

=> S HYDROLASE;S L3;S L5 OR L6;S SUBTILISIN;S L4;S L8,L9

L5 14370 HYDROLASE

L6 2169 L3

L7 14699 L5 OR L6

L8 4968 SUBTILISIN

L9 4122 L4

L10 6184 (L8 OR L9)

L11 14960 TRANSESTERIF?

=> S CYSTEINE OR CYS

63047 CYSTEINE
13663 CYS
L12 71205 CYSTEINE OR CYS

=> S L11,L12

L13 86120 (L11 OR L12)

=> S L11 AND L12

L14 45 L11 AND L12

=> S L10 AND L14

L15 4 L10 AND L14

=> S L10 AND L7

L16 101 L10 AND L7

=> S L7 AND L 14

955862 L
505026 14
306 L 14
(L(W)14)
L17 0 L7 AND L 14

=> S L7 AND L14

L18 2 L7 AND L14

=> S L15,L18

L19 4 (L15 OR L18)

=> S L14 NOT L19

L20 41 L14 NOT L19

=> D L19 1-4 CBIB ABS;D L20 1-41 CBIB ABS

L19 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2001 ACS
2000:335530 Document No. 132:344868 Chemically modified mutant serine hydrolases show improved catalytic activity and chiral selectivity. Jones, John Bryan; Dickman, Michael (Genencor International, Inc., USA). PCT Int. Appl. WO 2000028007 A2 20000518, 68 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US26586 19991109. PRIORITY: US 1998-PV107758 19981110; US 1998-PV113061 19981221.
AB This invention provides novel chem. modified mutant serine hydrolases that catalyze a transamidation and/or a transpeptidation and/or a ***transesterification*** reaction. The modified serine hydrolases have one or more amino acid residues in a subsite replaced with a ***cysteine*** , wherein the ***cysteine*** is modified by replacing the thiol hydrogen in the ***cysteine*** with a substituent group providing a thiol side chain comprising a moiety selected from the group consisting of a polar arom. substituent, an alkyl amino group with a pos. charge, and a glycoside. In particularly preferred embodiments, the substituents include an oxazolidinone, a C1-C15 alkyl amino group with a pos. charge, or a glycoside. Thus, covalent modification of Bacillus lentus ***subtilisin*** ***cysteine*** mutants with mandelate-based, oxozolidinone-based, or indanol-based chiral ligands causes remarkable changes in activity and specificity.

L19 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2001 ACS
1999:49990 Document No. 130:219776 Chemically modified mutants of ***subtilisin*** Bacillus lentus catalyze ***transesterification*** reactions better than wild type. Dickman, Michael; Lloyd, Richard C.; Jones, J. Bryan (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). Tetrahedron: Asymmetry, 9(23), 4099-4102 (English) 1998. CODEN: TASYE3. ISSN: 0957-4166. Publisher: Elsevier Science Ltd..
AB A combined site-directed mutagenesis and chem. modification strategy has been used to create superior enzyme catalysts for the resoln. of racemic primary and secondary alcs. using a ***transesterification*** reaction. The chem. modified mutant, N62C-S-CH3, of ***subtilisin***

Bacillus lentus catalyzes the ***transesterification*** of N-acetyl-L-phenylalanine vinyl ester with .beta.-branched primary alcs. faster than wild type. The ***cysteine*** mutant, M222C, of ***subtilisin*** Bacillus lentus gives higher yields (98% and 92% yields with 1-phenylethanol and 2-octanol, resp., vs. 19% and 10% for wild type) and better enantioselectivity than wild type when secondary alcs. are used.

L19 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2001 ACS
1994:503060 Document No. 121:103060 Engineering ***Subtilisin*** for Peptide Coupling: Studies on the Effects of Counterions and Site-Specific Modifications on the Stability and Specificity of the Enzyme. Sears, Pamela; Schuster, Matthias; Wang, Peng; Witte, Krista; Wong, Chi-Huey (Department of Chemistry, Scripps Research Institute, La Jolla, CA, 92037, USA). J. Am. Chem. Soc., 116(15), 6521-30 (English) 1994. CODEN: JACSAT. ISSN: 0002-7863.

AB Several variants derived from the thermostable ***subtilisin*** 8397 were made to create an enzyme that is more stable toward org. solvents or has a broader specificity for the P1' residue in amidation or is more effective for peptide segment ligation in aq. soln. To improve the stability in org. solvents, one of three surface charges was removed each time from 8397 to create the variants: Lys43 .fwdarw. Asn (K43N), Lys256 .fwdarw. Tyr (K256Y), and Asp181 .fwdarw. Asn (D181N). Although the stabilities of these variants in high concns. of hydrophilic org. solvents were higher than that of the wild-type enzyme, the D181N variant was less stable than the 8397 variant. It appears that removal of isolated surface charges does not necessarily improve the enzyme stability in polar org. solvents. A dramatic change of the enzyme stability in DMF was, however, obsd. in the presence of different counterions. ***Subtilisin*** BPN' lyophilized from Tris-HCl buffer (50 mM, pH 8.4) and suspended in DMF (the enzyme is partially sol.), for example, was completely inactivated in 30 min at 25.degree., while the enzyme still retained about 70% of the original activity in a week if lyophilized from sodium phosphate buffer (50 mM, pH 8.4) (the enzyme is completely insol. in DMF). In general, the enzyme lyophilized from org. buffers deactivates in DMF much faster than that from inorg. buffers. A similar counterion effect was obsd. with other variants. These studies suggest that subtilisins are very unstable when exposed directly to DMF; the stability is, however, markedly improved if the enzyme is protected by water or salts from contact with the solvent. To use subtilisins and variants in ***transesterification*** or aminolysis in org. solvents, water (3-30%) is usually present to have significant reactivity, and for ***transesterifications*** , it was found that a good rate and yield could be achieved in ethanol contg. 30% water. For use in peptide segment ligation in aq. soln., the active-site serine of ***subtilisin*** 8397/C206Q was converted chem. to ***cysteine*** , forming thiosubtilisin 8397/C206Q, and the aminolysis:hydrolysis ratio was several orders of magnitude higher than that for ***subtilisin*** BPN' and comparable to that for thiosubtilisin BPN'. The 8397 variant was also modified at the S1' site via M222A/Y217W mutations to broaden the P1' specificity.

L19 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2001 ACS
1988:607529 Document No. 109:207529 Preparation of enzymes having altered ***transesterification*** /hydrolysis rate ratios and nucleophile specificities. Arbige, Michael Vincent; Estell, David Aaron; Pepsin, Michael Jay; Poulose, Ayrookaran Joseph (Genencor, Inc., USA). Eur. Pat. Appl. EP 260105 A2 19880316, 11 pp. DESIGNATED STATES: R: DE, ES, FR, GB, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1987-307918 19870908. PRIORITY: US 1986-905363 19860909; US 1987-86869 19870821.

AB Enzymes with catalytic triads, e.g. serine or cystine hydrolases, are altered to change the ***transesterification*** rate/hydrolysis rate ratio and nucleophile specificity by altering amino acid residues within 15 .ANG. of the triad. [Phe-222] ***subtilisin*** BPN' has a ***transesterification*** /hydrolysis ratio (tyrosine Et ester substrate) 2.6-fold greater than that of the wild-type enzyme. When used to hydrolyze triacetin, this mutant showed a distinct preference for EtOH over H2O as the attacking nucleophile.

L20 ANSWER 1 OF 41 CAPLUS COPYRIGHT 2001 ACS
2000:359540 Document No. 133:116604 Glutathione as an essential factor for chaperon-mediated activation of lactonizing lipase (LipL) from Pseudomonas sp. 109. Tanaka, Junko; Nihira, Takuya; Yamada, Yasuhiro (Department of

Biotechnology, Graduate School of Engineering, Osaka University, Suita, 565-0871, Japan). J. Biochem. (Tokyo), 127(4), 597-601 (English) 2000. CODEN: JOBIAO. ISSN: 0021-924X. Publisher: Japanese Biochemical Society.

AB Pseudomonas sp. 109 produces a unique lipase (LipL) which efficiently catalyzes intramol. ***transesterification*** of .omega.-hydroxy-esters to form macrocyclic lactones. The prodn. of the enzymically active LipL requires a specific mol. chaperon (LimL protein) together with a low-Mr lipase-activation-factor (LAF) of unknown structure. From 50 g of Pseudomonas cells, 2.15 mg of LAF was purified as a sulfobenzofurazanyl deriv. after methanol extn., derivatization, and C18 reverse-phase HPLC. One-dimensional and two-dimensional 600 mHz 1H-NMR and fast atom bombardment mass spectrometry (FAB-MS) revealed that LAF is glutathione. Because several SH compds. (L- ***cysteine*** and mercaptoethanol) were similarly effective to native LAF in the activation of LipL, and because only LipL contains two cysteinyl residues forming an intramol. disulfide bond, it is concluded that the redn. of and reformation of the intramol. disulfide bond of LipL is essential to liberate free and fully active LipL.

L20 ANSWER 2 OF 41 CAPLUS COPYRIGHT 2001 ACS

2000:132390 Document No. 132:261720 Dissecting the chemistry of protein splicing and its applications. Noren, Christopher J.; Wang, Jimin; Perler, Francine B. (New England BioLabs, Inc., Beverly, MA, 01915, USA). Angew. Chem., Int. Ed., 39(3), 450-466 (English) 2000. CODEN: ACIEF5. ISSN: 1433-7851. Publisher: Wiley-VCH Verlag GmbH.

AB A review, with .apprx.95 refs. Inteins are internal polypeptide sequences that are posttranslationally excised from a protein precursor by a self-catalyzed protein-splicing reaction. The protein splicing domain (.apprxeq. 150 amino acids) activates cleavage of the peptide bonds at the N- and C-terminal splice junctions, with concomitant formation of a new peptide bond between the flanking polypeptides (exteins). The intein plus a single C-extein amino acid can break two peptide bonds, form a new peptide bond, cleave DNA, and initiate mobility of the intein gene. Nearly 100 inteins have been identified with Ser or ***Cys*** at the intein N terminus and the triad, His-(Asn/Gln)-(Ser/Thr/ ***Cys***), at the C-terminal splice junction. These conserved residues are known to participate in well-studied chem. reactions in other enzymes, which led to a flurry of proposed splicing mechanisms. This review focuses on the three-year period during which a combination of exptl. approaches revealed the steps of the protein-splicing pathway, disproving many proposed mechanisms while supporting others. The protein-splicing mechanism involves four coupled nucleophilic displacements: (1) an N .fwdarw. O(S) acyl shift of Ser/ ***Cys*** at the intein N terminus; (2) a ***transesterification*** reaction to form a branched intermediate with two N termini; (3) cyclization of the intein C-terminal Asn/Gln to release the intein; and (4) an O(S) .fwdarw. N acyl shift of Ser/Thr/ ***Cys*** to form a native peptide bond between the exteins. How the intein facilitates these reactions at the mol. level is only beginning to be elucidated. Understanding the mechanism of protein splicing has resulted in the development of a variety of intein-mediated protein-engineering applications, such as protein purifn., addn. of fluorescent biosensors, expression of cytotoxic proteins, protein semisynthesis, and segmental labeling of proteins for NMR anal. Inteins in pathogenic microorganisms provide new targets for drug discovery.

L20 ANSWER 3 OF 41 CAPLUS COPYRIGHT 2001 ACS

2000:120327 Document No. 132:305104 Reactivity of the ***Cysteine*** Residues in the Protein Splicing Active Center of the Mycobacterium tuberculosis RecA Intein. Shingledecker, Kaori; Jiang, Shu-qin; Paulus, Henry (Boston Biomedical Research Institute, Boston, MA, 02114, USA). Arch. Biochem. Biophys., 375(1), 138-144 (English) 2000. CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Academic Press.

AB Protein splicing involves the self-catalyzed excision of an intervening polypeptide segment, an intein, from a precursor protein. The first two steps in the protein splicing process lead to the formation of ester intermediates through nucleophilic attacks by the side chains of ***cysteine*** , serine, or threonine residues adjacent to the splice junctions. Since both nucleophilic residues in the Mycobacterium tuberculosis RecA intein are ***cysteine*** , their reactivities could be compared by sulfhydryl group titrn. This was accomplished by using fusion proteins contg. a truncated RecA intein modified by mutation to prevent protein splicing, in which the cysteines at the splice junctions were the only sulfhydryl groups. The ability to undergo hydroxylamine-induced cleavage at the upstream splice junction showed that the modified intein was not impaired in the ability to form ester

intermediates. Sulfhydryl titrn. with iodoacetamide, monitored by quantitating the residual thiols after reaction with a maleimide deriv. of biotin, revealed a striking difference in the apparent pKa values of the cysteines at the two splice junctions. The apparent pKa of the ***cysteine*** at the upstream splice junction, which initiates the N-S acyl rearrangement leading to the linear ester intermediate, was approx. 8.2, whereas that of the ***cysteine*** residue at the downstream splice junction, which initiates the ***transesterification*** reaction converting the linear ester to the branched ester intermediate, was about 5.8. This suggests that the ***transesterification*** step is facilitated by an unusually low pKa of the attacking thiol group. Comparison of the rates of cleavage of the linear ester intermediates derived from the M. tuberculosis RecA and the Saccharomyces cerevisiae VMA inteins by dithiothreitol and hydroxylamine revealed that the former reacted relatively more slowly with dithiothreitol, suggesting that the RecA intein has diverged in the course of evolution to react preferentially with thiolate anions and thus lacks the basic groups that may facilitate nucleophilic attack by thiols in other inteins. (c) 2000 Academic Press.

L20 ANSWER 4 OF 41 CAPLUS COPYRIGHT 2001 ACS
1999:788994 Document No. 132:64492 An improved synthesis of tert-butyl N.alpha.-tert-butoxycarbonyl-L-(S-trityl)cysteinate. Masiukiewicz, Elzbieta; Rzeszotarska, Barbara (Department of Organic Chemistry, University of Opole, Opole, 45-052, Pol.). Org. Prep. Proced. Int., 31(5), 571-572 (English) 1999. CODEN: OPPIAK. ISSN: 0030-4948. OTHER SOURCES: CASREACT 132:64492. Publisher: Organic Preparations and Procedures, Inc..

AB Tert-Bu N.alpha.-tert-butoxycarbonyl-L-(S-trityl)cysteinate, Boc-***Cys*** (Trt)-OtBu, was prepd. in high yield and high purity by esterification of H-***Cys*** (Trt)-OH with tert-Bu acetate using HClO4 as catalyst, followed by acylation with Boc2O in the presence of triethylamine. The product was obtained as well-shaped crystals in 80% yield (99.7% purity), following purifn. by column chromatog.

L20 ANSWER 5 OF 41 CAPLUS COPYRIGHT 2001 ACS
1998:757560 Document No. 130:135418 The chemical basis of protein splicing. Paulus, Henry (Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115, USA). Chem. Soc. Rev., 27(6), 375-386 (English) 1998. CODEN: CSRVBR. ISSN: 0306-0012. Publisher: Royal Society of Chemistry.

AB A review with 40 refs. Protein splicing is a recently discovered mechanism for the post-translational processing of proteins. It involves the self-catalyzed excision of an intervening polypeptide, the intein, from an inactive enzyme precursor and the formation of an active enzyme by joining the flanking regions by a peptide bond. Protein splicing occurs at a catalytic center that resides entirely within the intein. The catalyzed reactions include rearrangement of a peptide bond adjacent to ***cysteine*** or serine to yield a peptide ester, intramol. ***transesterification*** involving a second ***cysteine*** , serine, or threonine side chain to yield a branched protein, cyclization of an asparagine residue coupled to peptide bond cleavage to effect intein excision. This review discusses the mechanisms of these reactions and of similar reactions that underlie other types of protein rearrangements as well as the current state of knowledge on how these reactions are catalyzed.

L20 ANSWER 6 OF 41 CAPLUS COPYRIGHT 2001 ACS
1998:345166 Document No. 129:105558 Protein splicing: a novel form of gene expression and paradigm for self-catalyzed protein rearrangements. Paulus, Henry (Boston Biomed. Res. Inst., Boston, MA, 02114, USA). Pure Appl. Chem., 70(1), 1-8 (English) 1998. CODEN: PACHAS. ISSN: 0033-4545. Publisher: Blackwell Science Ltd..

AB A review with 29 refs. Protein splicing is one of the mechanisms by which genes that are interrupted by intervening sequences can produce functional proteins. It involves the self-catalyzed excision of an internal segment from an inactive precursor protein and the ligation of the flanking N- and C-terminal segments to yield an active involving the amino group of serine or ***cysteine*** to an ester bond. Such N-O or N-S acyl shifts are also the basis of other self-catalyzed protein rearrangements, which include the cleavage of hedgehog proteins and certain amidotransferases and the formation of pyruvoyl enzymes. Although N-O or N-S acyl rearrangements are thermodynamically unfavorable, their coupling to self-catalyzed irreversible steps drives the protein rearrangements to completion. In protein splicing, theses steps are intramol.

transesterification followed by asparagine cyclization. and peptide bond cleavage. All steps of protein splicing are catalyzed by the intervening sequence, which is a composite protein with sep. catalytic centers for protein splicing and DNA homing endonuclease activity. Expts. are in progress to study the structure and function of the catalytic center for protein splicing by the genetic dissection of the intervening sequence.

L20 ANSWER 7 OF 41 CAPLUS COPYRIGHT 2001 ACS
1998:129599 Document No. 128:214912 Crystal structure of human arylsulfatase A: The aldehyde function and the metal ion at the active site suggest a novel mechanism for sulfate ester hydrolysis. Lukatela, G.; Krauss, N.; Theis, K.; Selmer, T.; Gieselmann, V.; von Figura, K.; Saenger, W. (Institut fuer Kristallographie, Freie Universitaet Berlin, Berlin, D-14195, Germany). Biochemistry, 37(11), 3654-3664 (English) 1998. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.
AB Human lysosomal arylsulfatase A (ASA) is a prototype member of the sulfatase family. These enzymes require the posttranslational oxidn. of the -CH2SH group of a conserved ***cysteine*** to an aldehyde, yielding a formylglycine. Without this modification sulfatases are catalytically inactive, as revealed by a lysosomal storage disorder known as multiple sulfatase deficiency. The 2.1 .ANG. resoln. x-ray crystal structure shows an ASA homooctamer composed of a tetramer of dimers, (.alpha.2)4. The .alpha./.beta. fold of the monomer has significant structural analogy to another hydrolytic enzyme, the alk. phosphatase, and superposition of these two structures shows that the active centers are located in largely identical positions. The functionally essential formylglycine is located in a pos. charged pocket and acts as ligand to an octahedrally coordinated metal ion interpreted as Mg2+. The electron d. at the formylglycine suggests the presence of a 2-fold disordered aldehyde group with the possible contribution of an aldehyde hydrate, -CH(OH)2, with gem-hydroxyl groups. In the proposed catalytic mechanism, the aldehyde accepts a water mol. to form a hydrate. One of the two hydroxyl groups hydrolyzes the substrate sulfate ester via a ***transesterification*** step, resulting in a covalent intermediate. The second hydroxyl serves to eliminate sulfate under inversion of configuration through C-O cleavage and re-formation of the aldehyde. This study provides the structural basis for understanding a novel mechanism of ester hydrolysis and explains the functional importance of the unusually modified amino acid.

L20 ANSWER 8 OF 41 CAPLUS COPYRIGHT 2001 ACS
1997:127631 Document No. 126:222216 Histidine 265 is important for covalent catalysis by vaccinia topoisomerase and is conserved in all eukaryotic type I enzymes. Petersen, Birgitte O.; Shuman, Stewart (Mol. Biol. Program, Sloan-Kettering Inst., New York, NY, 10021, USA). J. Biol. Chem., 272(7), 3891-3896 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB Vaccinia topoisomerase catalyzes DNA cleavage and rejoining via ***transesterification*** to pentapyrimidine recognition site 5'-(C/T)CCTT .dwnarw. in duplex DNA. The proposed reaction mechanism involves general-base catalysis of the attack by active site nucleophile Tyr-274 on the scissile phosphodiester and general-acid catalysis of the expulsion of the 5'-deoxyribose oxygen on the leaving DNA strand. The pK.alpha. values suggest histidine and ***cysteine*** side chains as candidates for the roles of proton acceptor and donor, resp. To test this, we replaced each of the eight histidines and two cysteines of the vaccinia topoisomerase with alanine. Single mutants C100A and C211A and a double mutant C100A-C211A were fully active in DNA relaxation, indicating that a ***cysteine*** is not the general acid. Only one histidine mutation, H265A, affected enzyme activity. The rates of DNA relaxation, single-turnover strand cleavage, and single-turnover religation by H265A were 2 orders of magnitude lower than the wild-type rates. Yet the H265A mutation did not alter the dependence of the cleavage rate on pH, indicating that His-265 is not the general base. Replacing His-265 with glutamine or asparagine slowed DNA relaxation and single-turnover cleavage to about one-third of the wild-type rate. All three mutations, H265A, H265N, and H265Q, skewed the cleavage-religation equil. in favor of the covalently bound state. His-265 is strictly conserved in every member of the eukaryotic type I topoisomerase family.

L20 ANSWER 9 OF 41 CAPLUS COPYRIGHT 2001 ACS
1996:292010 Document No. 125:59016 Synthesis of cis-4-hydroxy-L-proline and its incorporation into biologically important peptides. Stavropoulos, George; Magafa, Vassiliki; Karagiannis, Kostas; Papaioannou, Dionissios

(Department Chemistry, University Patras, Patra, 260 10, Greece). Epitheor. Klin. Farmakol. Farmakokinet., Int. Ed., 9(2 and 3), 103-106 (English) 1995. CODEN: EFKEEB. ISSN: 1011-6583.

GI

/ Structure 7 in file .gra /

AB An efficient intramol. Mitsunobu reaction resulted in the conversion of trans-4-hydroxy-N-trityl-L-proline to 2-oxa-5-aza-bicyclo[2.2.1]heptan-3-one I. This lactone is a key intermediate in the synthesis of cis-4-hydroxy-L-proline (Hyp) and derivs. suitable for use in peptide synthesis. Methanolysis catalyzed by Ph3P-diethyl azodicarboxylate (DEAD) transformed the lactone into Ph3C-Hyp-OMe, while aminolysis in iso-Pr alc. gave the corresponding amide Ph3C-Hyp-NH2. Detritylation of lactone, ester and amide was affected by treatment with p-toluenesulfonic acid. On the other hand sapon. of the lactone provided Ph3C-Hyp-OH, which after O-benzylation and carboxy activation allowed the incorporation of Hyp into model peptides such as Trt- ***Cys*** (CPh3)-Hyp(CH2Ph)-Lys(CPh3)-Gly-NH2 and Trt-Hyp(CH2Ph)-Leu-Gly-NH2. Similar methodol. was applied to the TRH analog Glp-His(CPh3)-Hyp-OH, prepd. by solid-phase methods on the bulky and mild acid sensitive solid support 2-chlorotrityl resin, to convert it to Glp-His(CPh3)-Hyp lactone with inversion of configuration at C-4 of the Hyp residue. ***Transesterification*** of this lactone with MeOH/Ph3P-DEAD, followed by detritylation, provided the tripeptide ester Glp-His-Hyp-OMe which gave the corresponding amide and acid on aminolysis and sapon., while x-ray crystallog., FT-IR and 1H NMR techniques were used for structure identification of the thus prepd. compds.

L20 ANSWER 10 OF 41 CAPLUS COPYRIGHT 2001 ACS

1995:820925 Document No. 123:208908 Bilayer preparations of lipids in polar solvents as carriers for active agents. Carlsson, Anders; Hersloef, Bengt; Petrovic-kaellholm, Snezana (Karlshamns Lipidteknik AB, Swed.). PCT Int. Appl. WO 9520944 A1 19950810, 43 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-SE116 19950206. PRIORITY: SE 1994-368 19940204; SE 1994-2455 19940712.

AB The invention relates to a lipid-polar solvent bilayer prepn. consisting of 0.01-90%, preferably 0.1-50%, of a bilayer-forming material in a polar solvent, wherein the bilayer-forming material is a galactolipid material from cereals consisting of at least 50% digalactosyldiacylglycerols, and remainder being other polar lipids. The bilayer prepn. can be used as a carrier for an active substance in a pharmaceutical, cosmetic, food or agricultural product. Digalactosyldiacylglycerols were isolated from oat kernels and glutens and their fatty acid profile anal. was done by gas chromatog. after ***transesterification*** of the lipids to fatty acid Me ester. A gel was formulated contg. the galactolipids 22.1, hydrocortisone 1.2, propanol 16.1, and water 60.6%.

L20 ANSWER 11 OF 41 CAPLUS COPYRIGHT 2001 ACS

1994:185967 Document No. 120:185967 Role of the His- ***Cys*** finger of Moloney murine leukemia virus integrase protein in integration and disintegration. Jonsson, Colleen B.; Roth, Monica J. (Robert Wood Johnson Med. Sch., Univ. Med. Dent., Piscataway, NJ, 08854, USA). J. Virol., 67(9), 5562-71 (English) 1993. CODEN: JOVIAM. ISSN: 0022-538X.

AB Retroviral integrases mediate site-specific endonuclease and ***transesterification*** reactions in the absence of exogenous energy. The basis for the sequence specificity in these integrase-viral DNA recognition processes is unknown. Structural analogs of the disintegration substrate were made to analyze the disintegration reaction mechanism for the Moloney murine leukemia virus (M-MuLV) integrase (IN). Modifications in the target DNA portion of the disintegration substrate decreased enzymic activity, while substitution of the highly conserved CA in the viral long terminal repeat portion had no effect on activity. The role of the His- ***Cys*** finger region in catalysis was addressed by N-ethylmaleimide (NEM) modification of the ***cysteine*** residues of M-MuLV IN as well as by mutations. Both integration activities, 3' processing and strand transfer, were completely inhibited by NEM modification of M-MuLV IN, while disintegration activity was only

partially sensitive. However, structural analogs of the disintegration substrates that were modified in the target DNA and had the conserved CA removed were not active with NEM-treated M-MuLV IN. In addn., mutants made in the His- ***Cys*** region of M-MuLV IN were examd. and found to also be completely blocked in integration but not disintegration activity. These data suggest that the domains of M-MuLV IN that are required for the forward integration reaction substrate differ from those required for the reverse disintegration reaction substrate.

L20 ANSWER 12 OF 41 CAPLUS COPYRIGHT 2001 ACS
1993:517285 Document No. 119:117285 Preparation of benzoxathiaazabicyclododecines as novel DNA gyrase inhibitors. Arisawa, Mikio; Goetschi, Erwin; Kamiyama, Tsutomu; Masciadri, Raffaello; Shimada, Hisao; Watanabe, Junko; Hebeisen, Paul; Link, Helmut (Hoffmann-La Roche, F., und Co. A.-G., Switz.). PCT Int. Appl. WO 9218490 A1 19921029, 164 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-EP809 19920409. PRIORITY: EP 1991-106105 19910417.

GI

/ Structure 8 in file .gra /

AB A process for the prepn. of the title compds. I (X1 = S or SO, X2 = C(O) or C(S), R1 = H, alkyl, halogen, R2,R3 = H, alkyl, halogen, amino, acylamino, R4 = H, R5 = H, esterified carboxy or amidated carboxy, R6,R7 = H, alkyl, R8 = H, alkyl, esterified carboxy or amidated (thio)carboxy group) useful as antimicrobials, are prepd. E.g., 1.1 g of 3,5-diacetoxy-6-[(R)-2-((S)-2-(1-tert-butoyformamido)-3-methylbenzoic acid was added to dithiobis(4-tertbutyl-1-isopropylimidazole) and PPH3 (.74 g) to give tert-Bu (4R, 7S)-12,14-diacetoxy-1,3,4,5,6,7,8,10-octahydro-4-methoxy carbonyl-11-methyl-6,10-dioxo-9,2,5-benzoxa thiaazacyclododecine-7-carbamate as white crystals.

L20 ANSWER 13 OF 41 CAPLUS COPYRIGHT 2001 ACS
1990:515845 Document No. 113:115845 Suppression of the skin response to alantolactone in alantolactone-sensitized guinea pigs treated with N-acetyl-L-cysteinyl-L-alanine methyl ester. Chelli, Mario; Dupuis, Gilles; Evangelista, Stefano; Ginanneschi, Mauro; Meli, Alberto; Papini, Anna Maria; Rapi, Gianfranco (Dip. Chim. Org. "Ugo Schiff", Univ. Firenze, Florence, 50121, Italy). Eur. J. Med. Chem., 25(2), 107-15 (English) 1990. CODEN: EJMCA5. ISSN: 0223-5234.

GI

/ Structure 9 in file .gra /

AB Induction of a state of tolerance (hyposensitization) in alantolactone-sensitized guinea pigs was attempted by s.c. injections of dihydroalantolactonyl dipeptide I, Ac- ***Cys*** -Ala-OMe (II), or Ac- ***Cys*** -H (III). I was prepd. by addn. of Z- ***Cys*** -Ala-OMe (Z=PhCH2O2C) to alantolactone, followed by the removal of the Z group. Dipeptide II was obtained from Ac- ***Cys*** (CONHEt)-Ala-OMe (IV). Treatment of dipeptide IV with HBr-AcOH mixt. afforded mainly the S-acetyl deriv., from which dipeptide II was also obtained. Biol. assays showed that the alantolactone adduct I or III did not significantly modify the pos. skin response to alantolactone in alantolactone-sensitive guinea pigs. In marked contrast, dipeptide II significantly decreased the skin reaction to alantolactone tested at either 0.25 or 0.08 .mu.g. Control animals did not show skin responses to alantolactone after treatment with dipeptides I, II, or III. The data suggest that dipeptide II is an efficient and nontoxic tolerogen in the case of guinea pigs sensitized to alantolactone.

L20 ANSWER 14 OF 41 CAPLUS COPYRIGHT 2001 ACS
1990:76719 Document No. 112:76719 A .gamma.-lactam analog of the penems possessing antibacterial activity. Baldwin, Jack E.; Freeman, Richard T.; Lowe, Christopher; Schofield, Christopher J.; Lee, Eun (Dyson Perrins Lab., Oxford, OX1 3QY, UK). Tetrahedron, 45(14), 4537-50 (English) 1989. CODEN: TETRAB. ISSN: 0040-4020. OTHER SOURCES: CASREACT 112:76719.

GI

/ Structure 10 in file .gra /

AB The title compd. (I) was prepd. from (S)-PhCH2O2CNHCH(CO2CH2Ph)CH2CHO and D- ***cysteine*** . I has .apprx.5% of the bactericidal activity of penicillin G against Staphylococcus aureus. The satd. analog of I was inactive.

L20 ANSWER 15 OF 41 CAPLUS COPYRIGHT 2001 ACS
1989:529324 Document No. 111:129324 Presence of covalently attached fatty acids in rat apolipoprotein B via thiolester linkages. Kamanna, Vaijinath S.; Lee, Diana M. (Lipoprotein Atherosclerosis Res. Program, Oklahoma Med. Res. Found., Oklahoma City, OK, 73104, USA). Biochem. Biophys. Res. Commun., 162(3), 1508-14 (English) 1989. CODEN: BBRCA9. ISSN: 0006-291X.
AB Thiolester-linked lipids in rat apolipoprotein B (ApoB) were examd. by incubating reduced and carboxymethylated ApoB in 6M urea buffer with [14C]methylamine at pH 8.5, 30.degree.. It was obsd. that [14C]methylamine was covalently incorporated into ApoB, and there was a [14C]methylamine-modified product which was lipid in nature. After extn. with org. solvents, the [14C]methylamine-labeled product showed its Rf (relative mobility) on TLC to be similar to that of the synthetic N-Me fatty-acyl amide. After purifn. on TLC and ***transesterification*** with 3 N methanolic HCl, Me esters of C16:0, C18:0, and C18:1 fatty acids were identified by gas-liq. chromatog. These results suggest that rat ApoB, similar to human ApoB, contained covalently linked fatty acids through the high energy, labile thiolester bonds.

L20 ANSWER 16 OF 41 CAPLUS COPYRIGHT 2001 ACS
1989:439848 Document No. 111:39848 Synthesis of peptides containing S-(N-alkylcarbamoyl) ***cysteine*** residues, metabolites of N-alkylformamides in rodents and in humans. Threadgill, Michael D.; Gledhill, Adrian P. (Pharm. Sci. Inst., Aston Univ., Birmingham, B4 7ET, UK). J. Org. Chem., 54(12), 2940-9 (English) 1989. CODEN: JOCEAH. ISSN: 0022-3263. OTHER SOURCES: CASREACT 111:39848.
AB Hydrochloride salts of S-(N-methylcarbamoyl), S-(N-ethylcarbamoyl), and S-(N,N-dimethylcarbamoyl) derivs. of ***cysteine*** , N-acetylcysteine, and cysteinylglycine have been prepd. S-(N-Methylcarbamoyl)glutathione hydrochloride has also been synthesized. Protecting groups for amino and carboxylic acid functions were selected for their ability to solubilize the peptides in CH2Cl2, the slovent in which the thiols were treated with alkyl isocyanates and with Me2NCOCl. Removal of S-(amidomethyl) protecting groups using Hg(OAc)2 caused some loss of N-(tert-butoxycarbonyl) groups. Elimination of disulfide was evident during coupling of disulfide derivs. of ***cysteine*** using mixed anhydride methods but not with a carbodiimide coupling agent. Mixed disulfide protections were reductively cleaved by HS(CH2)3SH. Many of the deprotected S-carbamoyl amino acids and peptides are metabolites of the corresponding N-alkylformamides in rodents and in humans.

L20 ANSWER 17 OF 41 CAPLUS COPYRIGHT 2001 ACS
1988:108385 Document No. 108:108385 Identification of the thiol ester linked lipids in apolipoprotein B. Huang, Gang; Lee, Diana M.; Singh, Shyam (Health Sci. Cent., Univ. Oklahoma, Oklahoma City, OK, 73190, USA). Biochemistry, 27(5), 1395-400 (English) 1988. CODEN: BICHAW. ISSN: 0006-2960.
AB Human plasma low-d. lipoproteins (d. 1.032-1.043 g/mL) were totally delipidated. The reduced and carboxymethylated apolipoprotein B was incubated with 50 mM [14C]methylamine at pH 8.5 at 30.degree.. Covalent incorporation of [14C]methylamine was obsd. with concomitant generation of new SH groups, which could be blocked with [3H]- or [14C]iodoacetic acid. One class of [14C]methylamine-modified product was sepd. from the protein and was found to be lipid. Its Rf on TLC was similar to that of synthetic N-Me fatty acyl amides. After purifn. by TLC and ***transesterification*** in 3N methanolic HCl, Me esters of C16- and C18-fatty acids at a 1:1 ratio were identified by gas-liq. chromatog. The ***transesterification*** method was verified with known N-Me fatty acyl amides. Apparently, labile thiol ester-linked palmitate and stearate occur in apolipoprotein B. Under mild alk. conditions, the thiol ester bonds are broken by methylamine, forming N-Me fatty acyl amides and releasing new SH groups. Intramol. thiol ester bonds linked between ***cysteine*** side chains and acidic amino acid residues were also present.

L20 ANSWER 18 OF 41 CAPLUS COPYRIGHT 2001 ACS
1988:21721 Document No. 108:21721 Preparation of

dihydro(thiomethyl)pyridines as antihypertensives. Gandolfi, A. Carmelo; Frigerio, Marco; Spinelli, Silvano; Tofanetti, Odoardo; Tognella, Sergio (Boehringer Biochemia Robin S.p.A., Italy). PCT Int. Appl. WO 8700836 A1 19870212, 91 pp. DESIGNATED STATES: W: AU, BB, BG, BR, DK, FI, HU, JP, KP, KR, LK, MC, MG, MW, NO, RO, SD, SU; RW: AT, BE, CF, CG, CH, CM, DE, FR, GA, GB, IT, LU, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1986-EP445 19860729. PRIORITY: IT 1985-21876 19850806; IT 1986-20965 19860627; IT 1986-20966 19860627.

GI

/ Structure 11 in file .gra /

AB The title compds. [I; R1 = acyl, cyano, NO2, alkoxycarbonyl, H2NCO; R2 = (un)substituted aryl, heteroaryl; R3 = CO2R4; R4 = H, alkenyl, (un)substituted alkyl, aryl, aralkyl; X = S(O)nR5; R5 = H, acyl, (un)substituted alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heterocyclyl, alkoxyalkyl, alkylthioalkyl, thiuronium; n = 0-2] were prepd. as antihypertensives. Dihydropyridinedicarboxylate II (Y = Cl) (6 g) was refluxed with 1.2 g (H2N)2CS in EtOH to give 4.8 g dihydropyridinyisothiuronium salt II.HCl [Y = SC(:NH)NH2] (III). III had an IC50 of 2.2 .times. 10-7 M for inhibition of aortal-strip contraction and in rats 3.1 mg III/kg orally reduced blood pressure 45 mmHg.

L20 ANSWER 19 OF 41 CAPLUS COPYRIGHT 2001 ACS
1987:616127 Document No. 107:216127 Synthesis of esters by lipases. Lazar, G.; Weiss, A.; Schmid, R. D. (Dep. Biotechnol., Henkel K.-G.a.A., Duesseldorf, D-4000, Fed. Rep. Ger.). Proc. - World Conf. Emerging Technol. Fats Oils Ind., Meeting Date 1985, 346-54. Editor(s): Baldwin, A. Richard. Am. Oil Chem. Soc.: Champaign, Ill. (English) 1986. CODEN: 56DPAV.

AB Lipase is capable of esterification and ***transesterification*** reactions with a wide range of substrates. By using immobilization techniques, high operational stability of lipases can be achieved which renders the fraction cost of lipase favorable. However, in most cases, enzymic ester synthesis competes with simple chem. esterification procedures. As a consequence, biotechnol. methods are promising only where asym. carbon atoms are formed, labile esters have to be handled, or lipase selectivity allows for the prepn. of products which otherwise are difficult to obtain. Data are given on the esterification of fatty acids (oleic, stearic, palmitic, and 2-methylpentanoic acids) with various alcs. using lipases from different yeasts and fungi. The prepn. of glyceride esters is also considered.

L20 ANSWER 20 OF 41 CAPLUS COPYRIGHT 2001 ACS
1986:225210 Document No. 104:225210 Solid-phase synthesis of the human insulin B (5-9) analog with special rotary reactor. Koock, Soon Uoong; Hong, Nam Joo; Shin, Seung Dong (Dep. Chem., Korea Univ., 132, S. Korea). Han'guk Saenghwa Hakhoechi, 18(4), 410-16 (English) 1985. CODEN: KBCJAK. ISSN: 0368-4881.

AB Protected title peptide Me3CO2C-Phe-Gly- ***Cys*** (CH2Ph)-Phe-Gly-OMe was prepd. by the solid-phase method using an inexpensive app. consisting of a 60 mL capacity glass cylinder (5 .times. 6 cm) fritted filter disk embedded in the flask and a one-way 1.5 mm stopcock. The rotary reactor was used to avoid splashing any resin particles up on the sides of the vessel. All coupling steps were monitored by the ninhydrin procedure.

L20 ANSWER 21 OF 41 CAPLUS COPYRIGHT 2001 ACS
1985:471331 Document No. 103:71331 Acylamino oxo or hydroxy-substituted alkylamino thiazines and thiazepines. Weller, Harold N., III; Gordon, Eric M.; Karanewsky, Donald S.; Ryono, Denis E. (Squibb, E. R., and Sons, Inc. , USA). U.S. US 4512988 A 19850423, 16 pp. (English). CODEN: USXXAM. APPLICATION: US 1984-585058 19840301.

GI

/ Structure 12 in file .gra /

AB Antihypertensive (no data) thiazines and thiazepines I and II [R = H, alkyl, aminoalkyl, hydroxyalkyl, haloalkyl; R1 = H, alkyl, PhCH2, Ph2CH, Me3SiCH2CH2, salt forming ion, CHR7O2CR8 (R7 = H, alkyl, cycloalkyl, Ph;

R8 = R7, alkoxy, PhCH2, PhCH2CH2); R2 = R3(CH2)mCONHCH[(CH2)nR4]C(Z); R3 = (substituted) Ph, thienyl, furyl, pyridyl; R4 = R3, OH, NH2, SH, halo, indolyl, imidazolyl, alkylthio, guanidino, carbamoyl, cycloalkyl; m = 0-4; n = 1-4; Z = O, (H, OH); R5, R6 = H, alkyl, cycloalkylalkyl, R5R6 = benzo; o = 1, 2] were prepd. via inter- and intramol. cyclocondensations of ***cysteine*** derivs. Thus, cyclocondensation of N-phthaloyl-L- ***cysteine*** with PhCH:NCH2CO2Et gave thiazineacetate III as a mixt. of diastereomers, the (2S)-isomer of which was ***transesterified*** with Me3SiCH2CH2OH, deprotected, alkylated with (S)-PhCH2CH(NHBz)COCH2Cl and hydrolyzed to give [2S-[2.alpha.,5.alpha.(S)]]-thiazine IV.

L20 ANSWER 22 OF 41 CAPLUS COPYRIGHT 2001 ACS
1984:611189 Document No. 101:211189 Thiazine- and thiazepine-containing compounds. Karanewsky, Donald S. (Squibb, E. R., and Sons, Inc. , USA). U.S. US 4460579 A 19840717, 31 pp. (English). CODEN: USXXAM. APPLICATION: US 1983-470882 19830228.

GI

/ Structure 13 in file .gra /

AB Thiazine- and thiazepineacetates I [R, R3 = H, alkyl, Ph2CH, alkali metal ion, alk. earth metal ion, CHR4O2CR5; R1 = C1-10 alkyl, heteroaryl, heteroarylalkyl, cycloalkyl, cycloalkylalkyl, aminoalkyl, (un)substituted Ph, phenylalkyl, R6CONHCHR7CH2; R2 = H, alkyl, aminoalkyl, hydroxyalkyl, haloalkyl; R4 = H, alkyl, cycloalkyl, Ph; R5 = H, alkyl, alkoxy, cycloalkyl, Ph, PhCH2, PhCH2CH2; R6, R7 = alkyl, haloalkyl, cycloalkyl, cycloalkylalkyl, heteroaryl, heteroarylalkyl, (un)substituted Ph, phenylalkyl; X = CHR8, n = 1, 2; X = CHR8CHR9, o-C6H4, n = 1; R8, R9 = H, alkyl, cycloalkyl, cycloalkylalkyl, (un)substituted Ph, phenylalkyl], useful as antihypertensives (no data), were prepd. Thus, H2NCH2CO2Et.cntdot.HCl was condensed with BzH to give PhCH:NCH2CO2Et which was cyclocondensed with N-phthaloyl-L- ***cysteine*** , prepd. from N,N'-diphthaloyl-L-cystine, to give (R)-thiazineacetate II (R10 = phthalimido). Hydrazinolysis of the latter compd. gave II (R10 = H2N) which was treated with Ph(CH2)4P(O)(OEt)Cl to give II [R10 = Ph(CH2)4P(O)(OEt)NH]. This compd. was treated with Me3SiBr and LiOH to give phosphinylaminothiazineacetate III.

L20 ANSWER 23 OF 41 CAPLUS COPYRIGHT 2001 ACS
1984:210437 Document No. 100:210437 Antimicrobial disulfide prodrugs. Gilvarg, Charles; Kingsbury, William D. (SmithKline Beckman Corp., USA). U.S. US 4427582 A 19840124, 9 pp. (English). CODEN: USXXAM. APPLICATION: US 1982-386365 19820608.

AB H-Xn-NHCH(CO2H)CH2SSR [I; X = Ala, Orn, Lys, Phe; n = 1-5, R = residue of an antimicrobial mercaptan, e.g., 2-pyridyl, 2-pyridyl N-oxide, or 2-(2,6-dicarboxy-4-pyridylamino)ethyl] or their salts were prepd. as prodrugs. Thus, a mixt. of .beta.-(2-pyridyldithio)-L-alanine, Na2CO3, 1N NaOH, and H2O-MeCN was cooled to -10.degree., excess alanine N-carboxyanhydride in MeCN was added, and the mixt. was stirred for 3 h at 0.degree. to give I (Xn = Ala, R = 2-pyridyl). The latter (800 nmol) inhibited the growth of E. coli on seed agar plates (inhibition zone 15 nm, vs. 10-11 nm for 2-mercaptopyridine).

L20 ANSWER 24 OF 41 CAPLUS COPYRIGHT 2001 ACS
1984:98915 Document No. 100:98915 Irreversible inhibition of phosphotransacetylase by S-dimethylarsino-CoA. Duhr, Edward F.; Owens, Martha S.; Barden, Roland E. (Dep. Chem., Univ. Wyoming, Laramie, WY, 82071, USA). Biochim. Biophys. Acta, 749(1), 84-90 (English) 1983. CODEN: BBACAQ. ISSN: 0006-3002.

AB S-Dimethylarsino-CoA (I) was synthesized by acylation of CoA with dimethylchloroarsine. The new analog of acetyl-CoA was tested as an active-site-directed irreversible inhibitor of phosphotransacetylase (EC 2.3.1.8) (II), carnitine acetyltransferase (EC 2.3.1.7) (III), and citrate synthase (EC 4.1.3.7) (IV). Irreversible inhibition was obsd. only with II, which was derivatized via a simple bimol. process (k2 = 197 min-1 M-1). Acetyl-CoA provided complete substrate protection against the inactivation, whereas phosphate (a substrate) and desulfo-CoA (a competitive inhibitor) provided partial protection. The inactivation was not reversed by dithiothreitol. I was a linear competitive inhibitor vs. acetyl-CoA with both III (Ki = 41 .mu.M) and IV (Ki = 20 .mu.M). Chem. studies showed that I reacts with the SH of N.alpha.-acetylcysteine, but not with the side-chain functional groups of histidine and lysine. The nature of the chem. modification of ***cysteine*** was detd. by

investigating a model system. Thus, the chem. reaction between the thioarsenite linkage of S-dimethylarsinobenzylmercaptan and the SH of ***cysteine*** was shown to involve ***transesterification*** of the dimethylarsino group.

L20 ANSWER 25 OF 41 CAPLUS COPYRIGHT 2001 ACS
1983:558825 Document No. 99:158825 Synthesis of the mitogenic S-[2,3-bis(palmitoyloxy)propyl]-N-palmitoylpentapeptide from Escherichia coli lipoprotein. Wiesmueller, Karl Heinz; Bessler, Wolfgang; Jung, Guenther (Inst. Org. Chem., Univ. Tuebingen, Tuebingen, Fed. Rep. Ger.). Hoppe-Seyler's Z. Physiol. Chem., 364(5), 593-606 (English) 1983. CODEN: HSZPAZ. ISSN: 0018-4888.

GI

/ Structure 14 in file .gra /

AB The title peptide (I, Pal = palmitoyl) was prepd. by coupling ***cysteine*** II with H-Ser(CMe3)-Ser(CMe3)-Asn-Ala-OCMe3 (III) by CCC/1-hydroxybenzotriazole and deblocking the resulting protected peptide by CF3CO2H. Cystine di-tert-Bu ester was N-acylated with Pal-Cl and then reduced by dithioerythritol to give Pal-***Cys***-OCMe3, which was S-alkylated with BrCH2CH(OH)CH2OH to give ***cysteine*** IV (R = H), which was condensed with palmitic acid by DCC to give IV (R = Pal), which was de-tert-butylated to give II. III was prepd. from H-Ala-OCMe3 by stepwise coupling of 9-fluorenylmethoxycarbonyl (Fmoc) amino acids, using piperidine for Fmoc deblocking. The diastereomers of IV (R = Pal) with opposite configuration at the Pr C-2 atom were sepd. by silica gel chromatog.

L20 ANSWER 26 OF 41 CAPLUS COPYRIGHT 2001 ACS
1983:488559 Document No. 99:88559 Synthesis of a protected heptapeptide B14-20 of insulin B-chain by the solid-phase method. Schou, O.; Salem, E. M. (Danish Inst. Prot. Chem., Den.). Egypt. J. Chem., Volume Date 1981, 24(1-3), 173-8 (English) 1982. CODEN: EGJCA3. ISSN: 0367-0422.

GI

/ Structure 15 in file .gra /

AB Title peptide Boc-Ala-Leu-Tyr(BzlBr-2)-Leu-Val-***Cys***(Ztf)-Gly-OR [I; Boc = Me3CO2C, BzlBr-2 = CH2C6H4Br-2, Ztf = CH(CF3)NHCO2CH2Ph, R = H] (II) was prepd. by the solid-phase method. The protected peptide was cleaved from the resin by ***transesterification*** with Me2NCH2CH2OH/thallous ethoxide to give I (R = CH2CH2NMe2), which was sapond. to give II. Under more drastic conditions, the Ztf group was cleaved and dimer III formed.

L20 ANSWER 27 OF 41 CAPLUS COPYRIGHT 2001 ACS
1981:488168 Document No. 95:88168 Electron transport across polymeric membranes containing vitamin K1. Takeishi, Makoto; Inomata, Naokiyo; Hayama, Shigeru (Dep. Polym. Chem., Yamagata Univ., Yamagata, 992, Japan). Makromol. Chem., Rapid Commun., 2(5), 347-50 (English) 1981. CODEN: MCRCD4. ISSN: 0173-2803.

AB Oxidn.-redn. systems across polymeric membranes were studied. The membrane was a block copolymer prepd. by ***transesterification*** of di-Me terephthalate with 1,4-butanediol and poly(oxytetramethylene) of mol. wt. of .apprx.40,000. The polymer mixed with vitamin K1 and/or tetradecane in CHCl3 was spread on a glass plate and then the solvent (CHCl3) was evapd. and the film was dried under vacuum. The membrane was used in a 2-compartment cell, 1 side of which contained 0.1M FeCl3; the other, 0.1M L-***cysteine*** hydrochloride. Electron transport was indicated by measuring the potential of the aq. FeCl3, monitoring the redn. of Fe3+ to Fe2+.

L20 ANSWER 28 OF 41 CAPLUS COPYRIGHT 2001 ACS
1980:547846 Document No. 93:147846 Third component of human complement: appearance of a sulfhydryl group following chemical or enzymic inactivation. Prahl, James W.; Lorenz, Patrick E.; Schechter, Alan N.; Prah, James W.; Tack, Brian F. (Coll. Med., Univ. Utah, Salt Lake City, UT, 84132, USA). Biochemistry, 19(19), 4471-8 (English) 1980. CODEN: BICHAW. ISSN: 0006-2960.

AB Treatment of human C3 with hydroxylamine or hydrazine at physiol. pH and

ionic strength totally abrogates the intrinsic ability of this protein to sustain classical pathway-induced hemolysis of sheep red blood cells. Concomitant with the loss of this function the appearance of a single SH group can be followed by titrn. with the SH-specific reagents p-(chloromercuri)benzoate, [1-14C]iodoacetamide, 2,2'-dipyridyl disulfide, and 5,5'-dithiobis(2-nitrobenzoic acid). These reagents have also been used to follow the appearance of a free SH group on conversion of C3 to C3b with bovine trypsin. Autoradiog. of the electrophoretogram of sepd. .alpha., .alpha.'-, and .beta.-polypeptide chains of inactivated, [1-14C]carboxamidomethylated C3 samples has shown that the reactive SH group is present in the .alpha. chain of C3 and in the .alpha.' chain of C3b, resp. Digestion of the radiolabeled protein with porcine elastase has localized this SH group to a 28,000-dalton fragment of the .alpha. chain with immunochem. and functional reactivities of the C3d domain. Autoradiog. anal. of a hydrolyzate prepd. from radioalkylated C3 and subjected to high-voltage paper electrophoresis has shown the labeled amino acid to be [1-14C]-S-(carboxymethyl) ***cysteine*** . The susceptibility of native C3 to rapid and irreversible inactivation by nitrogen nucleophiles with the parallel appearance of a cysteinyl residue may indicate the presence of an internal thiol ester. The relation of the proposed thiol ester to the ability of nascent C3b to acylate cell surface components and carbohydrate is discussed within the context of a ***transesterification*** reaction.

L20 ANSWER 29 OF 41 CAPLUS COPYRIGHT 2001 ACS
1980:164294 Document No. 92:164294 Kinetic studies in peptide chemistry. Coupling, racemization and evaluation of methods useful for shortening coupling time. Kovacs, J.; Holleran, E. M.; Hui, K. Y. (Dep. Chem., St. John's Univ., Jamaica, NY, 11439, USA). J. Org. Chem., 45(6), 1060-5 (English) 1980. CODEN: JOCEAH. ISSN: 0022-3263.
AB Rate consts. were detd. for the racemization of R-Met-OR1 [I; R = PhCH2O2C (Z), Me3CO2C, Z-Gly; R1 = C6Cl5, C6F5, C6H4NO2-p, succinimido] with Et3N and for the peptide coupling reaction of I with H-Val-OMe. Methionine dipeptide active esters racemized through the usual 5(4H)-oxazolone route, which was in contrast to the enolization mechanism for the racemization of ***cysteine*** dipeptides. The time required for peptide coupling of a given % of amine component can be significantly reduced by using an excess of active ester.

L20 ANSWER 30 OF 41 CAPLUS COPYRIGHT 2001 ACS
1979:611814 Document No. 91:211814 Solid-phase synthesis of the protected octapeptide fragment (residues 1-8) of human insulin B-chain. Salem, Ezzeldin M.; Schou, O.; Christensen, T. (Tanning Res. Lab., Natl. Res. Cent., Cairo, Egypt). Indian J. Chem., Sect. B, 18B(2), 162-4 (English) 1979. CODEN: IJSBDB. ISSN: 0376-4699.
AB The title peptide Me3CO2C-Phe-Val-Asn(Mbh)-Gln(Mgh-His(Ztf)-Leu-***Cys*** (Ztf)-Gly-R (I; Mbh = 4,4'-dimethoxybenzhydryl, Ztf = 2,2,2-trifluoro-1-benzyloxycarbonylaminoethyl, R = OH) (II) was prepd. by the solid-phase method. I (R = O-resin) was prepd. by stepwise solid-phase couplings and then it was resin-cleaved by ***transesterification*** with HOCH2CH2NMe2 to give I (R = OCH2CH2NMe2), which was sapond. to give II.

L20 ANSWER 31 OF 41 CAPLUS COPYRIGHT 2001 ACS
1979:122020 Document No. 90:122020 The preparation of 2,2,2-trichloroethyl esters of some L-amino acids. Carson, John F. (Sci. Educ. Adm., WRRC, Berkeley, Calif., USA). Synthesis (1), 24-5 (English) 1979. CODEN: SYNTBF. ISSN: 0039-7881.
AB H-X-OCH2CCl3.p-MeC6H4SO3H [X = Gly, Ala, Val, Leu, Phe, Met, ***Cys*** (Me), ***Cys*** (CH2Ph)] were prepd. in 37-95% yields by the azeotropic distn. of H-X-OH, HOCH2CCl3, and p-MeC6H4SO3H in CCl4. Z-X1-OH (Z = PhCH2O2C, X1 = Val, Ala, Phe) were esterified with 2-hydroxypyridine by dicyclohexylcarbodiimide in pyridine at 0.degree. for 7 h to give the active esters, which were ***transesterified*** with HOCH2CCl3 to give Z-X1-OCH2CCl3, which were Z-deblocked by HBr/HOAc to give 58-66% H-X1-OCH2CCl3.Br.

L20 ANSWER 32 OF 41 CAPLUS COPYRIGHT 2001 ACS
1979:104340 Document No. 90:104340 A comparison between different coupling methods in the synthesis of the N-terminal nonapeptide of the sheep insulin-A chain by fragment condensation on a polymer support. Kaufmann, Klaus Dieter; Doelling, Rudolf (Sekt. Chem., Humboldt-Univ. Berlin, Berlin, E. Ger.). J. Prakt. Chem., 320(5), 814-24 (German) 1978. CODEN: JPCEAO. ISSN: 0021-8383.
AB H-Gly-O-resin underwent successive fragment condensations with BOC-

Cys (Bzl)-Ala-OH (BOC = Me3CO2C, Bzl = CH2Ph), BOC-Gl- ***Cys*** (Bzl)-OH, and Z-Gly-Ile-Val-Glu(OCMe3)-OH (Z = PhCH2O2C) by a variety of coupling methods to give Z-Gly-Ile-Val-Glu(OCMe3)-Glu- ***Cys*** (Bzl)-***Cys*** (Bzl)-Ala-Gly-OR (I, R = resin), which was ***transesterified*** with MeOH to give I (R = Me), the N-protected nonapeptide of sheep insulin A-chain. The best results for the solid-phase fragment couplings were obtained with dicyclohexylcarbodiimide/C6F5OH and the oxidn.-redn. condensation with 2,2'-dipyridyl disulfide/Ph3P.

L20 ANSWER 33 OF 41 CAPLUS COPYRIGHT 2001 ACS
1979:72445 Document No. 90:72445 Synthesis of pentafluorophenyl esters of amino acids and peptides using pentafluorophenyl trichloroacetate. Gudkov, A. T.; Shekhvatova, G. V. (Inst. Belka, Pushchino, USSR). Zh. Obshch. Khim., 48(9), 2146 (Russian) 1978. CODEN: ZOKHA4. ISSN: 0044-460X.

AB Treatment of 1 equiv. BOC- ***Cys*** (CH2Ph)-OH (BOC = Me3CO2C) and 1 equiv. Et3N with 10% excess Cl3CCO2C6F5 in a polar solvent gave 86% BOC-***Cys*** (CH2Ph)-OC6F5. PhCH2O2C-Gly-Ala-OC6F5 was similarly prepd. in 92% yield. These pentafluorophenyl esters were prepd. and used in active ester peptide coupling reactions without isolation.

L20 ANSWER 34 OF 41 CAPLUS COPYRIGHT 2001 ACS
1978:444187 Document No. 89:44187 Active esters in the formation of ester bonds between amino acids and polymeric supports. Bodanszky, Miklos; Fagan, Daniel T. (Dep. Chem., Case Western Reserve Univ., Cleveland, Ohio, USA). Int. J. Pept. Protein Res., 10(5), 375-9 (English) 1977. CODEN: IJPPC3. ISSN: 0367-8377.

AB BOC-Gly-OC6H4NO2-p (BOC = Me3CO2C), Z-Ile-OC6H4NO2-p (Z = PhCH2O2C), BOC-Pro-OSu (Su = succinimido), BOC-Leu-OC6Cl5, BOC-Asp(OCH2Ph)-OC6H2Cl3-2,4,5, and BOC- ***Cys*** (CH2Ph)-OC6H4NO2-o were ***transesterified*** with hydroxymethyl solid-phase resin by imidazole catalysis to give the corresponding N-protected amino acid esters with the hydroxymethyl polymer. Racemization was not obsd. BOC-Gly-OCH2Q (Q = polystyrene backbone) was used in the solid-phase prepn. of Z-Tyr(CH2Ph)-Ile-Gln-Asn-Lys(CO2CH2C6H4NO2-p)-Pro-Leu-Gly-OCH2Q (I) in which the individual amino acids were coupled to the peptidyl resin as active esters. I was cleaved by NH3/MeOH and the resulting peptide amide was deblocked by hydrogenation over Pd/C to give H-Tyr-Ile-Gln-Asn-Lys-Pro-Leu-Gly-NH2.

L20 ANSWER 35 OF 41 CAPLUS COPYRIGHT 2001 ACS
1978:424785 Document No. 89:24785 Removal of protected peptides from the Merrifield resin by potassium cyanide catalyzed ***transesterification*** . Moore, Graham; McMaster, Denis (Fac. Med., Univ. Calgary, Calgary, Alberta, Can.). Int. J. Pept. Protein Res., 11(2), 140-8 (English) 1978. CODEN: IJPPC3. ISSN: 0367-8377.

AB Protected amino acids and peptides were cleaved from the Merrifield resin by KCN-catalyzed ***transesterification*** with dry MeOH, EtOH, or PhCH2OH for 24 h at room temp. to give the appropriate amino acid or peptide ester. Racemization was not obsd. in the ***transesterification*** of Z-Ala-Val-resin (Z = PhCH2O2C) with PhCH2OH in the presence of KCN to give Z-Ala-Val-OCH2Ph. Z- ***Cys*** (CH2Ph)-Pro-Arg(SO2C6H4Me-p)-(CH2Ph)-Tyr(CH2Ph)-Ile-Gln-Asn- ***Cys*** (CH2Ph)-Gly-R (I, R = resin) was treated with PhCH2OH contg. KCN to give I (R = OCH2Ph) which was amidated, deblocked, and cyclized to give arginine-vasotocin. ***Transesterification*** in 95% aq. MeOH can be used for releasing the C-terminal free peptide from the resin, although this cannot be used for peptides which contain CO2H side chains protected as benzyl esters. Also, when the C-terminal amino acid is sterically hindered, the sapon. step is slow and an increase in racemization may result.

L20 ANSWER 36 OF 41 CAPLUS COPYRIGHT 2001 ACS
1978:90011 Document No. 88:90011 A new approach for esterification of amino acids. Jain, J. C.; Sharma, I. K.; Sahni, M. K.; Gupta, K. C.; Mathur, N. K. (Dep. Chem., Univ. Jodhpur, Jodhpur, India). Indian J. Chem., Sect. B, 15(8), 766-7 (English) 1977. CODEN: IJSBDB.

AB The Et esters of glycine, alanine, leucine, tyrosine, serine, ***cysteine*** , hydroxyproline, and tryptophan as well as the di-Et esters of aspartic and glutamic acids were prepd. by esterifying the corresponding amino acid with HC(OEt)3 (I) in EtOH; I acts as a H2O scavanger as well as a ***transesterification*** agent. Alanine, leucine, lysine, and serine were esterified using sulfonic acid resin (H+ form) as a catalyst and anhyd. CaSO4 as a H2O scavenger.

L20 ANSWER 37 OF 41 CAPLUS COPYRIGHT 2001 ACS
1976:122284 Document No. 84:122284 Synthesis of peptides by fragment condensation on a solid support. II. Scheme for preparation of 4,8-disubstituted vasopressins evaluated on 8-arginine-vasopressin. Larsson, Lars E.; Melin, Per; Ragnarsson, Ulf (Biokemiska Inst., Uppsala Univ., Uppsala, Swed.). Int. J. Pept. Protein Res., 8(1), 39-44 (English) 1976. CODEN: IJPPC3.

AB 8-Arginine-vasopressin, which exhibited full pressor activity, was prepd. from the fragments, PhCH2O2C- ***Cys*** (CH2Ph)-Tyr(CH2Ph)-Phe and Me3CO2C-Asn(Mbh)- ***Cys*** (CH2Ph)-Pro (Mbh = 4,4'-dimethoxybenzhydryl) which were prepd. conventionally with the carboxyl group protected as benzyl esters, followed by ***transesterification*** with 2-dimethylaminoethanol and hydrolysis. Racemization was avoided in the coupling step with the fragment contg. a C-terminal phenylalanine by adding N-hydroxysuccinimide. Final purifn. was effected by ion-exchange chromatog.

L20 ANSWER 38 OF 41 CAPLUS COPYRIGHT 2001 ACS
1975:98370 Document No. 82:98370 Fundamental experiments in solid-phase peptide synthesis. Beyerman, H. C. (Lab. Org. Chem., Tech. Hogesch. Delft, Delft, Neth.). Prog. Pept. Res., [Proc. Am. Pept. Symp.], 2nd, Meeting Date 1970, 25-34. Editor(s): Lande, Saul. Gordon and Breach: New York, N. Y. (English) 1972. CODEN: 29USAB.

AB PhCH2O2C- ***Cys*** (CH2Ph)-Tyr(CH2Ph)-Ile-Glu-Asn- ***Cys*** (CH2Ph)-Pro-Leu-Gly-OMe was prepd. in 70% overall yield by the solid phase method followed by ***transesterification*** from the polymer with 1N MeOH and N-methylpiperidine at room temp. ***Transesterification*** of Merrifield resin bound PhCH2O2C-Pro-Leu-Gly was studied with MeOH, EtOH, and Me2CHOH in various tertiary amines.

L20 ANSWER 39 OF 41 CAPLUS COPYRIGHT 2001 ACS
1971:100403 Document No. 74:100403 N-Carboxyanhydrides of amino acids and peptide synthesis. Peptides of N.epsilon.-benzyloxycarbonyl-L-lysine. Skalaban, T. D.; Nazimov, I. M.; Pankova, S. S.; Zvonkova, E. N.; Evstigneeva, R. P.; Preobrazhenskii, N. A. (Mosk. Inst. Tonkoi Khim. Tekhnol. im. Lomonosova, Moscow, USSR). Zh. Org. Khim., 7(1), 47-51 (Russian) 1971. CODEN: ZORKAE.

AB The reaction of N-.alpha.-carboxyanhydride of N.epsilon.-benzyloxycarbonyl-L-lysine (M. Bergman et al., 1935) with L-alanine at 0.degree. gave N.epsilon.-benzyloxycarbonyl-L-lysyl-L-alanine (I) which was tested as a reagent for the peptide synthesis. The condensation of I with N-carboxyanhydride of L-phenylalanine (M. Sela et al. 1955) gave L-phenylalanyl-N.epsilon.-benzyloxycarbonyl-L-lysyl-L-alanine. The condensation of I with tert-butoxycarbonyl azide gave N.alpha.-tert-butoxycarbonyl-N.epsilon.-benzyloxycarbonyl-L-lysyl-L-alanine. The ***transesterification*** of I with PhCH2OH gave N.epsilon.-benzyloxycarbonyl-L-lysyl-L-alanyl benzyl ether hydrochloride which was condensed with S(2,2-dicarbethoxyethyl)-N-formyl-L- ***cysteine*** to give formyl-S-(2,2-dicarbethoxyethyl-L-cysteinyl-N.epsilon.-carbobenzoxy-L-lysyl-L-alanyl benzyl ether.

L20 ANSWER 40 OF 41 CAPLUS COPYRIGHT 2001 ACS
1970:96798 Document No. 72:96798 Chromophore-protein bonds in phycocyanin. Crespi, Henry L.; Smith, Ursula H. (Chem. Div., Argonne Nat. Lab., Argonne, Ill., USA). Phytochemistry, 9(1), 205-12 (English) 1970. CODEN: PYTCAS.

AB Peptides formed by the proteolysis of C-phycocyanin were purified by thin-layer chromatog. and analyzed for amino acid content. The data suggest that phycocyanobilin is linked to apophycocyanin through two bonds, one an ester bond involving the carboxyl group of an aspartic acid side-chain and the hydroxyl group of the enol form of ring A of the bilin, and the other a thio-ether type of bond from a ***cysteine*** side-chain to the bilin side-chain at position 2, a linkage analogous to that in cytochrome c. Liberation of phycocyanobilin from apophycocyanin by alcs. would involve ***transesterification*** at the ester bond followed by the formation of the keto form of ring A and elimination of the ***cysteine*** side-chain.

L20 ANSWER 41 OF 41 CAPLUS COPYRIGHT 2001 ACS
1969:461746 Document No. 71:61746 Alcoholysis of the Merrifield-type peptide-polymer bond with an anion-exchange resin. Pereira, Wilfred; Close, Virginia A.; Jellum, E.; Patton, Walter; Halpern, Berthold (Med. Center, Stanford Univ., Stanford, Calif., USA). Aust. J. Chem., 22(6), 1337-40 (English) 1969. CODEN: AJCHAS.

AB The protected peptide esters, BOC-L-Leu-L- ***Cys*** (S-Bzl)-L-Ala-OMe, BOC-L-Ala-L-Leu-L-Phe-OMe, BOC-L-Met-L-Phe-Gly-L-Leu-L-Ala-OMe, BOC-Gly-(.epsilon.-Z)-L-Lys-L-Phe-Gly-L-Leu-L-Ala-OMe, R-L-Val-L-Ala-OMe (R = dimedonyl), BOC-L-Try-L-Phe-L-Pro-L-Ala-OMe, Z-Gly-L-Val-(OBzl)-L-Thr-OMe, BOC-(OBzl)-L-Tyr-Gly-OMe, BOC-L-Phe-L-Ala-(OBzl)-L-Ser-OMe, BOC-L-Asn-L-Ala-OMe, BOC-.epsilon.-Z-L-Lys-Gly-OMe, BOC-L-Ala-L-Glu(.gamma.-OMe)-L-Ala-OMe, BOC(Nim-Bzl)-L-His-L-Phe-OMe, BOC-L-Asp(.beta.-OMe)-L-Ala-OMe, BOC-L-Val-Gly-L-Asp(.beta.-OMe)-OMe, and N-Z-L-Pro-OBu-tert (where BOC = tert-butoxycarbonyl, Bzl = benzyl, and Z = benzyloxycarbonyl) are prepd. by the solid-phase ***transesterification*** technique with a yield of 50-70%. The peptides generally required further purification.

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=> E JONES J/AU
=> S E3,E10,E11,E18,E19

           112 "JONES J"/AU
            86 "JONES J B"/AU
            13 "JONES J B JR"/AU
           160 "JONES J BRYAN"/AU
             3 "JONES J BRYN"/AU
L21        374 ("JONES J"/AU OR "JONES J B"/AU OR "JONES J B JR"/AU OR "JONES
               J BRYAN"/AU OR "JONES J BRYN"/AU)

=> E JONES JOHN/AU
=> S E3,E8,E9,E14

            20 "JONES JOHN"/AU
             3 "JONES JOHN B"/AU
            11 "JONES JOHN B JR"/AU
            18 "JONES JOHN BRYAN"/AU
L22         52 ("JONES JOHN"/AU OR "JONES JOHN B"/AU OR "JONES JOHN B JR"/AU
               OR "JONES JOHN BRYAN"/AU)

=> S L21,L22

L23        426 (L21 OR L22)

=> E DICKMAN M/AU
=> S E3-E7,E12-E17

            14 "DICKMAN M"/AU
            11 "DICKMAN M B"/AU
            13 "DICKMAN M D"/AU
             2 "DICKMAN M H"/AU
             1 "DICKMAN M L"/AU
            10 "DICKMAN MICHAEL"/AU
             2 "DICKMAN MICHAEL D"/AU
            44 "DICKMAN MICHAEL H"/AU
             1 "DICKMAN MICHAEL HARRY"/AU
             1 "DICKMAN MICHAEL K"/AU
             1 "DICKMAN MICHAEL PHILIP"/AU
L24        100 ("DICKMAN M"/AU OR "DICKMAN M B"/AU OR "DICKMAN M D"/AU OR "DICK
               MAN M H"/AU OR "DICKMAN M L"/AU OR "DICKMAN MICHAEL"/AU OR "DICK
               MAN MICHAEL D"/AU OR "DICKMAN MICHAEL H"/AU OR "DICKMAN MICHAEL
               HARRY"/AU OR "DICKMAN MICHAEL K"/AU OR "DICKMAN MICHAEL PHILIP"/
               AU)

=> S L24,L23

L25        522 (L24 OR L23)

=> S L25 AND (L5,L8)

L26         43 L25 AND ((L5 OR L8))

=> S L26 NOT L14

L27         41 L26 NOT L14

=> S L27 AND L12

L28         17 L27 AND L12
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=> D 1-17 CBIB ABS

L28 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2001 ACS
2000:564490 Document No. 133:346449 Covalent modification of ***subtilisin*** Bacillus lentus ***Cysteine*** mutants with enantiomerically pure chiral auxiliaries causes remarkable changes in activity. ***Dickman, M.*** ; ***Jones, J. B.*** (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). Bioorg. Med. Chem., 8(8), 1957-1968 (English) 2000. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB Methanethiosulfonate reagents may be used to introduce virtually unlimited structural modifications in enzymes via reaction with the thiol group of ***cysteine*** . The covalent coupling of enantiomerically pure (R) and (S) chiral auxiliary methanethiosulfonate ligands to ***cysteine*** mutants of ***subtilisin*** Bacillus lentus induces spectacular changes in catalytic activity between diastereomeric enzymes. Amidase and esterase kinetic assays using a low substrate approxn. were used to establish kcat/KM values for the chem. modified mutants (CMMs), and up to 3-fold differences in activity were found between diastereomeric enzymes. Changing the length of the carbon chain linking the Ph or benzyl oxazolidinone ligand to the mutant N62C by a methylene unit reverses which diastereomeric enzyme is more active. Similarly, changing from a Ph to benzyl oxazolidinone ligand at S166C reverses which diastereomeric enzyme is more active. Chiral modifications at S166C and L217C give CMMs having both high esterase kcat/KM's and high esterase to amidase ratios with large differences between diastereomeric enzymes. :.

L28 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2001 ACS
2000:488710 Document No. 133:277977 Controlled site-selective protein glycosylation for precise glycan structure-catalytic activity relationships. Davis, B. G.; Lloyd, R. C.; ***Jones, J. B.*** (Department of Chemistry, University of Durham, Durham, DH1 3LE, UK). Bioorg. Med. Chem., 8(7), 1527-1535 (English) 2000. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB Glycoproteins occur naturally as complex mixts. of differently glycosylated forms which are difficult to sep. To explore their individual properties, there is a need for homogeneous sources of carbohydrate-protein conjugates and this has recently prompted us to develop a novel method for the site-selective glycosylation of proteins. The potential of the method was illustrated by site-selective glycosylations of ***subtilisin*** Bacillus lentus (SBL) as a model protein. A representative library of mono- and disaccharide MTS reagents were synthesized from their parent carbohydrates and used to modify ***cysteine*** mutants of SBL at positions 62 in the S2 site, 156 and 166 in the S1 site and 217 in the S1' site. These were the first examples of prepns. of homogeneous neoglycoproteins in which both the site of glycosylation and structure of the introduced glycan were predetd. The scope of this versatile method was expanded further through the combined use of peracetylated MTS reagents and careful pH adjustment to introduce glycans contg. different nos. of acetate groups. This method provides a highly controlled and versatile route that is virtually unlimited in the scope of the sites and glycans that may be conjugated, and opens up hitherto inaccessible opportunities for the systematic detn. of the properties of glycosylated proteins. This potential has been clearly demonstrated by the detn. of detailed glycan structure-hydrolytic activity relationships for SBL. The 48 glycosylated CMMs formed display kcat/KM values that range from 1.1-fold higher than WT to 7-fold lower than WT. The anomeric stereochem. of the glycans introduced modulates changes in kcat/KM upon acetylation. At positions 62 and 217 acetylation enhances the activity of .alpha.-glycosylated CMMs but decreases that of .beta.-glycosylated. This trend is reversed at position 166 where, in contrast, acetylation enhances the kcat/KMs of .beta.-glycosylated CMMs but decreases those of .alpha.-glycosylated. Consistent with its surface exposed nature changes at position 156 are more modest, but still allow control of activity, particularly through glycosylation with disaccharide lactose.

L28 ANSWER 3 OF 17 CAPLUS COPYRIGHT 2001 ACS
2000:441949 Document No. 133:70717 Chemical modification of enzymes with methanethiosulfonate reagents for adding multiple charges and altering specificity and/or activity. Davis, Benjamin G.; ***Jones, John*** *** Bryan*** ; Bott, Richard R. (Genencor International, Inc., USA). PCT Int. Appl. WO 2000037658 A2 20000629, 93 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,

DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US30362 19991220. PRIORITY: US 1998-PV113130 19981221; US 1999-467536 19991220.

AB A method and reagent kit for altering specificity of enzymes by chem. modification using methanethiosulfonate reagents combined with genetic engineering based site-directed mutagenesis, is disclosed. One or more amino acid residues of enzymes are replaced by ***cysteine*** residues, where the ***cysteine*** residues are modified by replacing the thiol hydrogen in the ***cysteine*** residues with a substituent group providing a thiol side chain comprising a multiply charged moiety. Preferred enzymes are a serine ***hydrolase*** or a protease such as Bacillus lentus ***subtilisin*** , cellulase, amylase, lactase, or lipase, and the amino acid replacement occurs in the binding site. Preferred amino acids for replacement by ***cysteine*** are asparagine, leucine, methionine, or serine. In case of a trypsin-type serine protease, it also includes tyrosine, and glutamine, and for alpha/beta serine ***hydrolase*** such as Candida antarctica lipase, threonine, valine, isoleucine, and alanine. Neg. charges can be introduced by sulfonatoethyl thiol, 4-carboxybutyl thiol, 3,5-dicarboxybenzyl thiol, 3,3-dicarboxybutyl thiol, and 3,3,4-tricarboxybutyl thiol. Pos. charges can be introduced by aminoethyl thiol, 2-(trimethylammonium)ethyl thiol, 4,4-bis(aminomethyl)-3-oxo-hexyl thiol, and 2,2-bis(aminomethyl)-3-aminopropyl thiol. Those multiply charges moiety may be either a dendrimer or a polymer. A method of assaying for a preferred enzyme and detg. the catalytic efficiency of an enzyme by detg. the degree of stain removal from the material is also claimed. Use of detergent as component of enzyme-contg. compn. is claimed.

L28 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2001 ACS

2000:184008 Document No. 133:4956 Glycomethanethiosulfonates: powerful reagents for protein glycosylation. Davis, Benjamin G.; Maughan, Michael A. T.; Green, Martin P.; Ullman, Astrid; ***Jones, J. Bryan*** (Department of Chemistry, University of Durham, Durham, DH1 3LE, UK). Tetrahedron: Asymmetry, 11(1), 245-262 (English) 2000. CODEN: TASYE3. ISSN: 0957-4166. Publisher: Elsevier Science Ltd..

AB Twelve novel glycomethanethiosulfonate (glyco-MTS) protein glycosylation reagents have been prepd. Their use in a controlled site-selective glycosylation strategy that combines site-directed mutagenesis with chem. modification allows protein glycosylation with concomitant control of (i) site, (ii) carbohydrate, (iii) anomeric stereochem., (iv) sugar to protein spacer arm nature and (v) degree of glycan protection. The ability of these highly selective and yet reactive reagents has been illustrated by the introduction of D-glucosyl and N-Ac-D-glucosaminyl residues to both external and hindered internal sites in a model protein, the serine protease enzyme ***subtilisin*** Bacillus lentus (SBL), using gluco-MTS and N-Ac-glucosamine-MTS. Mol. modeling studies provide a rationale for the strikingly different effects of these reagents on the properties of the protein despite differing only in the nature of their C-2 substituents.

L28 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2001 ACS

2000:34887 Document No. 132:89792 Neoglycoproteins and their preparation by reacting ***cysteine*** -containing proteins mutants with glycosyl thiosulfonate. ***Jones, J. Bryan*** ; Davis, Benjamin G. (Genencor International, Inc., USA). PCT Int. Appl. WO 2000001712 A2 20000113, 86 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US15138 19990702. PRIORITY: US 1998-91687 19980702; US 1999-131446 19990428.

AB The present invention relates to a chem. modified mutant protein including a ***cysteine*** residue substituted for a residue other than a ***cysteine*** in a precursor protein, the substituted ***cysteine*** residue being subsequently modified by reacting the ***cysteine*** residue with a glycosylated thiosulfonate. Also, a method of producing

the chem. modified mutant protein is provided. The present invention also relates to a glycosylated methanethiosulfonate. Another aspect of the present invention is a method of modifying the functional characteristics of a protein including providing a protein and reacting the protein with a glycosylated methanethiosulfonate reagent under conditions effective to produce a glycoprotein with altered functional characteristics as compared to the protein. In addn., the present invention relates to methods of detg. the structure-function relationships of chem. modified mutant proteins. Thus, a no. of glycosyl methanethiosulfonates were synthesized and reacted with the N62C, the S156C, the S166C, or the L217C mutants of Bacillus lentus ***subtilisin*** and the resulting neoglycoproteins were characterized. Thus, the L217C mutant was reacted with 2,3,4,6-tetra-O-acetyl-.beta.-D-glucopyranosyl methanethiosulfonate to prep. a monoglucosylated enzyme contg. 3 acetyl groups. This deriv. had a kcat/KM that was 8-fold greater than that of the wild-type enzyme. Addnl. there was an improvement in specificity for ester vs. amide hydrolysis: the ratio (kcat/KM)esterase/(kcat/KM)amidase was 17.2-fold greater than that of the wild-type. This modified enzyme may find use in enzymic peptide synthesis.

L28 ANSWER 6 OF 17 CAPLUS COPYRIGHT 2001 ACS
2000:9421 Document No. 132:148462 Altering the specificity of ***subtilisin*** Bacillus lentus through the introduction of positive charge at single amino acid sites. Davis, Benjamin G.; Khumtaveeporn, Kanjai; Bott, Richard R.; ***Jones, J. Bryan*** (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). Bioorg. Med. Chem., 7(11), 2303-2311 (English) 1999. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB The use of methanethiosulfonates as thiol-specific modifying reagents in the strategy of combined site-directed mutagenesis and chem. modification allows virtually unlimited opportunities for creating new protein surface environments. As a consequence of our interest in electrostatic manipulation as a means of tailoring enzyme activity and specificity, we have recently adopted this approach for the controlled incorporation of multiple neg. charges at single sites in the representative serine protease, ***subtilisin*** Bacillus lentus (SBL). We now describe the use of this strategy to introduce multiple pos. charges. A series of mono-, di- and triammonium methanethiosulfonates were synthesized and used to modify ***cysteine*** mutants of SBL at positions 62 in the S2 site, 156 and 166 in the S1 site and 217 in the S1' site. Kinetic parameters for these chem. modified mutants (CMM) enzymes were detd. at pH 8.6. The presence of up to three pos. charges in the S1, S1' and S2 subsites of SBL resulted in up to 77-fold lowered activity, possibly due to interference with the histidinium ion formed in the transition state of the hydrolytic reactions catalyzed.

L28 ANSWER 7 OF 17 CAPLUS COPYRIGHT 2001 ACS
2000:9420 Document No. 132:134059 The controlled introduction of multiple negative charge at single amino acid sites in ***subtilisin*** Bacillus lentus. Davis, Benjamin G.; Shang, Xiao; DeSantis, Grace; Bott, Richard R.; ***Jones, J. Bryan*** (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). Bioorg. Med. Chem., 7(11), 2293-2301 (English) 1999. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB The use of methanethiosulfonates as thiol-specific modifying reagents in the strategy of combined site-directed mutagenesis and chem. modification allows virtually unlimited opportunities for creating new protein surface environments. As a consequence of our interest in electrostatic manipulation as a means of tailoring enzyme activity and specificity, the authors have adopted this approach for the controlled incorporation of multiple neg. charges at single sites in the representative serine protease, ***subtilisin*** Bacillus lentus (SBL). A series of mono-, di- and triacidic acid methanethiosulfonates were synthesized and used to modify ***cysteine*** mutants of SBL at positions 62 in the S2 site, 156 and 166 in the S1 site and 217 in the S1' site. Kinetic parameters for these chem. modified mutant (CMM) enzymes were detd. at pH 8.6 under conditions which ensured complete ionization of the unnatural amino acid side-chains introduced. The presence of up to three neg. charges in the S1, S1' and S2 subsites of SBL resulted in up to 11-fold lowered activity, possibly due to interference with oxyanion stabilization of the transition state of the hydrolytic reactions catalyzed. Each unit increase in neg. charge resulted in a raising of Km and a redn. of kcat. However, no upper limit was obsd. for increases in Km, whereas decreases in kcat reached a limiting value. Comparison with sterically similar but uncharged CMMs revealed that electrostatic effects of neg. charges at positions 62, 156

and 217 are detrimental, but are beneficial at position 166. These results indicate that the ground-state binding of SBL to the std. substrate, Suc-AAPF-pNA, to SBL is reduced, but without drastic attenuation of catalytic efficiency, and show that SBL tolerates high levels of charge at single sites.

L28 ANSWER 8 OF 17 CAPLUS COPYRIGHT 2001 ACS
1999:577505 Document No. 131:334004 Toward Tailoring the Specificity of the S1 Pocket of ***Subtilisin*** B. lentus: Chemical Modification of Mutant Enzymes as a Strategy for Removing Specificity Limitations. DeSantis, Grace; Shang, Xiao; ***Jones, J. B.*** (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). Biochemistry, 38(40), 13391-13397 (English) 1999. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB In both protein chem. studies and org. synthesis applications, it is desirable to have available a toolbox of inexpensive proteases with high selectivity and diverse substrate preferences. Toward this goal, we have generated a series of chem. modified mutant enzymes (CMMs) of ***subtilisin*** B. lentus (SBL) possessing expanded S1 pocket specificity. Wild-type SBL shows a marked preference for substrates with large hydrophobic P1 residues, such as the large Phe P1 residue of the std. suc-AAPF-pNA substrate. To confer more universal P1 specificity on S1, a strategy of chem. modification in combination with site-directed mutagenesis was applied. For example, WT-SBL does not readily accept small uncharged P1 residues such as the -CH3 side chain of alanine. Accordingly, with a view to creating a S1 pocket that would be of reduced vol. providing a better fit for small P1 side chains, a large cyclohexyl group was introduced by the CMM approach at position S166C with the aim of partially filling up the S1 pocket. The S166C-S-CH2-c-C6H11 CMM thus created showed a 2-fold improvement in kcat/KM with the suc-AAPA-pNA substrate and a 51-fold improvement in suc-AAPA-pNA/suc-AAPF-pNA selectivity relative to WT-SBL. Furthermore, WT-SBL does not readily accept pos. or neg. charged P1 residues. Therefore, to improve SBL's specificity toward pos. and neg. charged P1 residues, we applied the CMM methodol. to introduce complementary neg. and pos. charged groups, resp., at position S166C in S1. A series of mono-, di-, and tri-neg. charged CMMs were generated and all showed improved kcat/KMs with the pos. charged P1 residue contg. substrate, suc-AAPR-pNA. Furthermore, virtually arithmetic improvements in kcat/KM were exhibited with increasing no. of neg. charges on the S166C-R side chain. These increases culminated in a 9-fold improvement in kcat/KM for the suc-AAPR-pNA substrate and a 61-fold improvement in suc-AAPR-pNA/suc-AAPF-pNA selectivity compared to WT-SBL for the tri-neg. charged S166C-S-CH2CH2C(COO-)3 CMM. Conversely, the pos. charged S166C-S-CH2CH2NH3+ CMM generated showed a 19-fold improvement in kcat/KM for the suc-AAPE-pNA substrate and a 54-fold improvement in suc-AAPE-pNA/suc-AAPF-pNA selectivity relative to WT-SBL.

L28 ANSWER 9 OF 17 CAPLUS COPYRIGHT 2001 ACS
1999:487229 Document No. 131:102548 Modified enzymes and their use for peptide synthesis. ***Jones, J. Bryan*** (Genencor International, Inc., USA). PCT Int. Appl. WO 9937324 A1 19990729, 51 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US1413 19990121. PRIORITY: US 1998-PV72351 19980123; US 1998-PV72265 19980123.

AB Peptide synthesis was carried by using modified enzymes having high esterase and low amidase activity. One or more amino acid residues from an enzyme is replaced by ***cysteine*** residue(s), where at least some of the ***cysteine*** residues are modified by replacing thiol hydrogen with a thiol side chain to form a modified enzyme. A ***cysteine*** mutant derived from ***subtilisin*** Bacillus lentus was modified with alkyl methanethiosulfonate reagents and applied to the synthesis of dipeptides.

L28 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2001 ACS
1999:487228 Document No. 131:99258 Chemically modified mutant enzymes, methods for producing and screening them, and their use as detergent and feed additives and for textile treatment. ***Jones, J. Bryan*** ; Plettner, Erika (Genencor International, Inc., USA). PCT Int. Appl. WO 9937323 A1 19990729, 30 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ,

BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.
(English). CODEN: PIXXD2. APPLICATION: WO 1999-US1230 19990121.
PRIORITY: US 1998-PV72266 19980123.

AB The present invention relates to method for screening chem. modified mutant enzymes for amidase and/or esterase activity. This method includes providing a chem. modified mutant enzyme with a substrate for an amidase and/or a substrate for an esterase and detg. whether the chem. modified mutant enzyme exhibits amidase and/or esterase activity. The present invention also relates to chem. modified mutant enzymes and a method for producing them where one or more amino acid residues from an enzyme are replaced by ***cysteine*** residues, and the ***cysteine*** residues are modified by replacing at least some of the thiol hydrogen in the ***cysteine*** residue with a thiol side chain to form the chem. modified mutant enzyme. The thiol side chain is selected from the group consisting of -SCH2(p-CH3-C6H4), -SCH2(p-OCH3-C6H4), -SCH2(p-CF3-C6H4), and -SCH2(2,4-diNO2-C6H3). The invention is demonstrated with Bacillus lentus ***subtilisin*** . After creating N62C, S166C, and L217C mutants, the newly created ***Cys*** residues were reacted with a series of phenylmethyl methanethiosulfonates. Some of the resulting derivs., esp. the mutants reacted with MeSO2SCH2(p-CO2H-C6H4), had a favorably increased esterase:amidase ratio.

L28 ANSWER 11 OF 17 CAPLUS COPYRIGHT 2001 ACS

1999:485967 Document No. 131:268900 Probing the altered specificity and catalytic properties of mutant ***subtilisin*** chemically modified at position S156C and S166C in the S1 pocket. DeSantis, Grace; ***Jones,*** *** J. Bryan*** (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). Bioorg. Med. Chem., 7(7), 1381-1387 (English) 1999. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB A series of chem. modified mutants (CMMs) of ***subtilisin*** B. lentus (SBL) were generated employing the combination of site-directed mutagenesis and chem. modification. This strategy entails the mutation of a selected active site residue to ***cysteine*** and its subsequent modification with a methanethiosulfonate reagent CH3SO2S-R, where R may be infinitely variable. The present study was undertaken to evaluate the changes in specificity and pH-activity profiles that could be induced by modification of S156C and S166C in the S1 pocket of SBL with a representative range of side chain modifications, namely R=CH3, CH2C6H5, CH2CH2NH3+ and CH2CH2SO3-. The side chain of S156C is surface exposed and well solvated while that of S166C points into the pocket. Kinetic evaluation of the CMMs with suc-AAPF-pNA as substrate showed that the kcat/KMs changed very little for the S156C CMMs, but varied by up to 11-fold for the S166C CMMs. The pH-activity profiles were also detd., and showed that a neg. or pos. charged side chain modification increased or decreased resp., the pKa of the catalytic triad histidine for both modification sites but with more dramatic changes for the interior pointing S166C than for the solvent exposed S156C site. As an addnl. probe of altered specificity, inhibition of the CMMs by a representative series of 5 boronic acid transition state analog inhibitors was detd. The Kis obsd. ranged from a 3.5-fold improvement over the WT value, to a 12-fold decrease in binding. Overall, greater variability in all the parameters measured, activity, pKa, and boronic acid binding resulted from modification at the inward pointing 166 site than at the solvent-exposed 156 site.

L28 ANSWER 12 OF 17 CAPLUS COPYRIGHT 2001 ACS

1999:298728 Document No. 131:70395 Modulation of Esterase and Amidase Activity of ***Subtilisin*** Bacillus lentus by Chemical Modification of ***Cysteine*** Mutants. Plettner, Erika; DeSantis, Grace; Stabile, Michele R.; ***Jones, J. Bryan*** (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). J. Am. Chem. Soc., 121(21), 4977-4981 (English) 1999. CODEN: JACSAT. ISSN: 0002-7863. Publisher: American Chemical Society.

AB For synthetic applications of proteases, such as for peptide coupling, a combination of high esterase and low amidase activities is required. While achieving such specificity has been a long-standing goal, the decreases in amidase activity achieved to date have often also been accompanied by decreases in esterase activity. In the current study, a strategy of combined site-directed mutagenesis and chem. modification was applied to the serine protease ***subtilisin*** Bacillus lentus (SBL)

to improve its esterase-over-amidase specificity. Using the crystal structure of SBL as a guide, the N62, L217, S166, and M222 active site residues were chosen for mutagenesis to ***cysteine*** and subsequent modification by alkyl methanethiosulfonate reagents. An initial rapid, combinatorial screen of the chem. modified mutant enzymes (CMMs) generated and, of their parent ***cysteine*** mutants, identified 25 promising candidates which were then subjected to detailed kinetic evaluations. Of these CMM and mutant enzymes, 20 exhibited an improvement, of up to 52-fold, in esterase-over-amidase activity compared to the wild type (WT). Furthermore, these increased esterase-to-amidase ratios were not gained at the expense of esterase activity, which was improved up to 3-fold higher than that of the WT in abs. terms. The general success of this approach is evident from the fact that, of the 25 CMMs and ***cysteine*** mutants evaluated, 19 displayed higher esterase activity than the WT, whereas only 3 had better than WT amidase activity.

L28 ANSWER 13 OF 17 CAPLUS COPYRIGHT 2001 ACS
1998:756618 Document No. 130:110474 Controlled Site-Selective Glycosylation of Proteins by a Combined Site-Directed Mutagenesis and Chemical Modification Approach. Davis, Benjamin G.; Lloyd, Richard C.; ***Jones,*** *** J. Bryan*** (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). J. Org. Chem., 63(26), 9614-9615 (English) 1998. CODEN: JOCEAH. ISSN: 0022-3263. Publisher: American Chemical Society.

AB Site-directed mutagenesis combined with chem. modification provides a general method that allows for both regio- and glycan-specific glycosylation of proteins. The strategy involves the introduction of ***cysteine*** at preselected positions and then reaction of its thiol residue with glycomethanethiosulfonate reagents. Four different sites of ***subtilisin*** Bacillus lentus (SBL) were mutated to ***cysteine*** (SBL-N62C, -S156C, -S166C, -L217C) and glycosylated using a series of protected and unprotected mono- and disaccharide methanethiosulfonates. Through adjustment of pH and appropriate selection of the glycosylation site, differently acetylated glycoforms of SBL were prepd.

L28 ANSWER 14 OF 17 CAPLUS COPYRIGHT 2001 ACS
1998:630356 Document No. 129:341164 A combinatorial approach to chemical modification of ***subtilisin*** Bacillus lentus. Plettner, Erika; Khumtaveeporn, Kanjai; Shang, Xiao; ***Jones, J. Bryan*** (Department Chemistry, University Toronto, Toronto, ON, M5S 3H6, Can.). Bioorg. Med. Chem. Lett., 8(17), 2291-2296 (English) 1998. CODEN: BMCLE8. ISSN: 0960-894X. Publisher: Elsevier Science Ltd..

AB The reaction between methanethiosulfonate reagents and ***cysteine*** mutants of ***subtilisin*** is quant. and can be used to prep. chem. modified mutant enzymes (CMMs) with novel properties. The virtually unrestricted structural variations possible for CMMs presents a preparative and screening challenge. To address this, a rapid combinatorial method for prepg. and screening the activities of CMMs has been developed.

L28 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2001 ACS
1998:275181 Document No. 128:305580 Site-directed mutagenesis combined with chemical modification as a strategy for altering the specificity of the S1 and S1' pockets of ***subtilisin*** Bacillus lentus. DeSantis, Grace; Berglund, Per; Stabile, Michele R.; Gold, Marvin; ***Jones, J. Bryan*** (Departments of Chemistry and of Molecular and Medical Genetics, University of Toronto, Toronto, ON, M5S 3H6, Can.). Biochemistry, 37(17), 5968-5973 (English) 1998. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB By combining site-directed mutagenesis with chem. modification, the S1 and S1' pocket specificity of ***subtilisin*** Bacillus lentus (SBL) was altered through the incorporation of unnatural amino acid moieties, in the following manner: WT .fwdarw. Cysmutant + H3CSO2SR .fwdarw. ***Cys*** -SR, where R may be infinitely variable. A paradigm between extent of activity changes and surface exposure of the modified residue has emerged. Modification of M222C, a buried residue in the S1' pocket of SBL, caused dramatic changes in kcat/Km, of an up to 122-fold decrease, while modification of S166C, which is located at the bottom of the S1 pocket and is partially surface exposed, effected more modest activity changes. Introduction of a pos. charge at S166C does not alter kcat/Km, whereas the introduction of a neg. charge results in lowered activity, possibly due to electrostatic interference with oxyanion stabilization. Activity is virtually unaltered upon modification of S156C, which is located toward the bottom of the S1 pocket and surface exposed and whose side chain is solvated. An unexpected structure-activity relationship was revealed for S166C-SR enzymes in that the pattern of activity changes obsd. with

increasing steric size of R was not monotonic. Mol. modeling anal. was used to analyze this unprecedented structure-activity relationship and revealed that the position of the .beta.-carbon of Cys166 modulates binding of the P1 residue of the AAPF product inhibitor.

L28 ANSWER 16 OF 17 CAPLUS COPYRIGHT 2001 ACS
1997:324853 Document No. 127:30808 Chemical modification of ***cysteine*** mutants of ***subtilisin*** Bacillus lentus can create better catalysts than the wild-type enzyme. Berglund, Per; DeSantis, Grace; Stabile, Michele R.; Shang, Xiao; Gold, Marvin; Bott, Richard R.; Graycar, Thomas P.; Lau, Tony Hing; Mitchinson, Colin; ***Jones, J. Bryan*** (Departments of Chemistry and of Molecular and Medical Genetics, University of Toronto, Toronto, ON, M5S 3H6, Can.). J. Am. Chem. Soc., 119(22), 5265-5266 (English) 1997. CODEN: JACSAT. ISSN: 0002-7863. Publisher: American Chemical Society.

AB The ***Cys*** side-chains of the N62C and L217C site-directed mutants of ***subtilisin*** Bacillus lentus (SBL) were chem. modified with 11 structurally varied, polar and nonpolar, methanethiosulfonate reagents to generate 22 chem. modified mutant enzymes, of which 10 surpassed the catalytic activity of their wild-type parent by up to >3 fold. This combined site-directed mutagenesis-chem. modification strategy offers virtually unlimited possibilities for creating novel enzymes possessing unnatural amino acid side-chains.

L28 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2001 ACS
1996:741314 Document No. 126:28523 Altering the specificity of ***subtilisin*** B. lentus by combining site-directed mutagenesis and chemical modification. Berglund, Per; Stabile, Michele R.; Gold, Marvin; ***Jones, J. Bryan*** (Dep. Chem. Mol. Med. Genetics, Univ. Toronto, Toronto, ON, M5S 3H6, Can.). Bioorg. Med. Chem. Lett., 6(21), 2507-2512 (English) 1996. CODEN: BMCLE8. ISSN: 0960-894X. Publisher: Elsevier.

AB The thiol side chain of the M222C mutant of the ***subtilisin*** from Bacillus lentus (SBL) has been chem. modified by methyl-, aminoethyl-, and sulfonatoethylthiosulfonate reagents. Introduction of charged residues into the active site of the enzyme reduced the catalytic efficiency with Suc-AAPF-pNA as the substrate, but resulted in better binding of sterically demanding boronic acid inhibitors.